In vitro study of the effect of an essential oil and a delmopinol mouth rinse on dental plaque bacteria

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ABSTRACT

Context: Mouthrinses are used, by many of our patients, as a complement to daily dental hygiene routine. The use of a toothbrush and an interproximal cleaning method may not be enough to control dental plaque. Essential oils and delmopinol mouth rinses are effective for the prevention of dental caries and gingivitis. To study the effect of an essential oil and a delmopinol mouth rinse on dental plaque bacteria, an *in vitro* study was developed.
Aims: The objective of this study was to determine the antibacterial activity of an essential oil and a delmopinol mouth rinse on *Streptococcus mutans*, *Lactobacilli*, and aerobic and anaerobic dental plaque nonspecific bacteria.
Design: Samples of human dental plaque were collected from consenting participants and bacteria isolated. Disk-diffusion tests were performed to obtain the minimum concentration of the mouth rinses necessary to inhibit bacterial growth. The ability of the commercial mouth rinses to inhibit bacterial growth was studied in comparison to a positive control (0.2% chlorhexidine) and a negative laboratorial control (sterilized water).
Results: The minimum inhibitory concentration was found to be inferior to the commercial essential oils and delmopinol mouth rinses concentrations. Delmopinol and essential oils have significant antibacterial properties shown *in vitro* only for aerobic bacteria, and for *S. mutans*, *Lactobacillus*, and anaerobic bacteria, the results were not statistically significant.
Conclusions: Essential oils and chlorhexidine are statistically similar and better than delmopinol for aerobic bacteria growth inhibition. For the other bacteria, essential oils and delmopinol are not statistically promising. Results show that essential oils only may help patients to maintain good oral health as a complement to daily brushing and interproximal cleaning.

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Essential oils are effective to prevent gingivitis and reduce dental plaque and are considered by the Food and Drug Administration (FDA) to be safe for human use.[1]

Essential oils have the ability to destroy the cell membrane and inhibit bacterial enzymatic activity; they are also able to prevent bacteria adhesion to the existing biofilm and reduce bacteria multiplication.[2,3] In addition, essential oils present a bactericidal effect,[4] causing 78.7% of bacteria to be nonvital after a 60 s exposure.[5]

Delmopinol mouth rinses

Delmopinol has anti-inflammatory properties[6] and prevents gingivitis.[6,7] The FDA approved it in 2005. Its efficacy is based on the ability to interfere with the bacterial matrix formation, inhibit the adhesion of bacteria,[8] cause a loose biofilm that is easier to remove.[6,9,10]

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The objective of this study was to determine the minimum inhibitory concentration (MIC) and the inhibition of bacterial growth of an essential oil and a delmopinol mouth rinse on *Streptococcus mutans*, *Lactobacilli*, and aerobic and anaerobic dental plaque (unspecified bacteria).

MATERIALS AND METHODS

Dental plaque samples were collected from patients at the Dental Hygiene Clinic (Faculdade de Medicina Dentária da Universidade de Lisboa) after approval of the Lisbon Dental School Ethics Committee and a signature of informed consent. Selection of patients was performed at the Lisbon Dental Hygiene Clinic by approaching individuals and asking them to participate voluntarily in this study. The eligibility criteria included being over 18 years of age, patients at the dental hygiene clinic, and patients who had not taken antibiotics in the previous 3 months. Furthermore, patients should not be users of mouth rinses. Patients were included in the study after signature was obtained on informed consent form; all patients from the Lisbon Dental School Clinic were eligible. Inclusion criteria required for the patient was to have at least 6 teeth in two quadrants, to have a gingival index lower than 3, and to have dental plaque present on teeth surfaces. Exclusion criteria were the previous use of a mouth rinse, below 18 years of age, having had a dental hygiene appointment in the previous 6 months, presence of extensive caries and fractured teeth, and not under antibiotic medication in the past 3 months before the study.

Laboratorial work was developed at the microbiology laboratory at Instituto Piaget – Campus Universitário de Almada.

Dental plaque was collected from two quadrants with a minimum of 6 teeth present. In one of the quadrants, supragingival samples were collected from the buccal surface of every tooth with a swab (BBL™ CultureSwab Plus™, Becton Dickinson, NJ, USA), and subgingival samples were collected from the other quadrant, using sterile absorbent paper points (Spik, Viannini Dental Industry, Grassina, Italy), size ISO 45, placed in the gingival sulcci for 30 s after removal of supragingival plaque with a curette.

An essential oil mouth rinse (Listerine Cool Mint®) and a delmopinol mouth rinse (Decapinol®) were tested as experimental products. A positive control of 0.2% chlorhexidine (Corsodyl®) and a negative control of sterilized water were used. Further, dilutions of the experimental products (100%, 50%, 25%, 12.5%, 6.25%, 3.12%, 1.56%, 0.78%, 0.39%, 0.19%) were tested to determine the MIC of bacterial growth.

For specimen preparation, swabs were placed in a 10 ml sterilized Ringer solution and dispersed in a vortex for 30 s at maximum speed (Heidolph–Reax Top, Schwabach, Germany). Samples were then diluted using a pipette (Calibra digital, Socorex, Lausanne, Switzerland) to place 1 ml of the Ringer solution with the dispersed sample in a test tube with 9 ml of sterilized Ringer solution, resulting in a tenfold dilution. This diluted solution was used to cultivate Petri dishes using specific culture media for *S. mutans*, *Lactobacillus*, and unspecific dental plaque.

For *S. mutans* culture, bacteria were first isolated using a selective media, Agar Mitis Salivarius (Difco™, Franklin Lakes, NJ, USA), with addition of bacitracin and sucrose. The media were sterilized for 15 min at 121°C and 20 ml was placed in each Petri dish. After inoculation of the dishes with 0.5 ml of the diluted Ringer solution, the samples were placed in anaerobic conditions for 48 h at 37°C, in an incubator (Incudigit, JP Selecta, Barcelona, Spain).

Bacteria with *S. mutans* characteristics were then identified using biochemical tests, such as Gram coloration, mannitol fermentation test, hemolytic reaction test, and catalase test.

For *Lactobacillus* culture, diluted samples were placed in a selective media, Agar Rogosa SL (Scharlau, Barcelona, Spain) added with glacial acetic acid. Twenty milliliters of the media was placed in each Petri dish for bacteria growth in anaerobic conditions for 72 h at 37°C.

For unspecific aerobic and anaerobic dental plaque bacteria culture, diluted samples were placed in 15 ml of nonselective media, brain heart Infusion Agar (Scharlau, Barcelona, Spain), sterilized at 120°C for 15 min, in each Petri dish. To obtain aerobic bacteria, dishes were placed in the incubator for 24 h at 37°C. For anaerobic bacteria, dishes were placed in anaerobic conditions for the same time and temperature.

Determination of the minimum inhibitory concentration

After isolation of *S. mutans*, *Lactobacillus*, and aerobic and anaerobic dental plaque nonspecific bacteria, a disk-diffusion test was used to determine the MIC of the essential oils and delmopinol mouth rinses. The disk-diffusion test uses 6 mm absorbent paper circles, with the test product diluted in several concentrations, to determine the minimal concentration able to inhibit visible bacterial growth. This test is useful to determine the effect of antiseptics in bacteria and should be performed at least in three Petri dishes with samples of bacteria.[11]

After isolation, bacteria were placed in a nutrient broth for bacterial growth (Nutrient Broth M002-Himedia Labs, Mumbai, India), using different test tubes for different bacteria. Each test tube was placed at 37°C for a minimum of 4 h under appropriate conditions for each bacterial species. After this period, bacteria were placed in Petri dishes with brain–heart infusion to grow. Each Petri dish had five absorbent paper disks with five dilutions of each

of the experimental mouth rinses, resulting in two dishes for each of the mouth rinses, one with the concentrations: 100%, 50%, 25%, 12.5%, and 6.25%, and the other dish with the concentrations: 3.12%, 1.56%, 0.78%, 0.39%, and 0.19%. The Petri dishes were incubated for 24 h at 37°C for *S. mutans*, *Lactobacillus*, and unspecific aerobic and anaerobic bacteria. After this time, the inhibition of bacterial growth was observed by the formation of halos with no visible bacteria, which were measured in mm.

Determination of inhibition of bacterial growth

To determine the inhibition of bacterial growth, besides the experimental mouth rinses, essential oils and delmopinol, a positive control (0.2% chlorhexidine) and a negative control (sterilized water) were also used. The disk-diffusion test was performed as described above. Inhibition of bacterial growth was determined by measuring the inhibition halos.

Data analysis

MIC was determined as the lowest concentration value where inhibition was observed.

For the determination of the inhibition of bacterial growth, parametric and nonparametric statistical methods were used according to the distribution of data.

Parametric methods were used when data followed a normal distribution verified using the Kolmogorov–Smirnov test. To compare two variables, the *t*-test was applied, and to compare more than two variables, the one-way ANOVA test was used.

For nonparametric data, the comparison of two variables was performed with the Mann–Whitney U-test; for more than two variables, the Kruskal–Wallis test was chosen. The significance level was set at $P = 0.05$.

RESULTS

Determination of the minimum inhibitory concentration

For every type of bacteria studied, the MIC was lower than the commercial product (concentration = 100%) available to the consumer. Results are shown in Table 1.

Determination of the inhibition of bacterial growth

Mean values, in millimeters, of growth inhibition halos, are presented in Table 2 for *S. mutans*, *Lactobacillus*, and aerobic and anaerobic bacteria colonies using the essential oils, delmopinol, and 0.2% chlorhexidine mouth rinses. *P* values are also presented. Sterilized water did not produce any bacterial growth inhibition.

Table 1: Minimum inhibitory concentration using a disk-diffusion test

	Essential oils (%)	Delmopinol (%)
Streptococcus mutans	1.56	0.78
Lactobacillus	3.12	1.56
Aerobic bacteria	6.25	6.25
Anaerobic bacteria	6.25	3.12

There was a statistically different result, among tested products, only for the aerobic bacteria growth inhibition. Individual comparisons among the products are presented in Table 3.

The data analysis shows that there are no differences between chlorhexidine and essential oils mouth rinses, and it also shows that delmopinol mouth rinse is statistically different from the chlorhexidine and essential oils mouth rinses.

DISCUSSION

The present study found that, under laboratory conditions using isolated bacteria (not in a biofilm), essential oils and delmopinol mouth rinses have MICs much smaller that the commercial products concentration.

This may result from the higher susceptibility of isolated bacteria to antiseptic products than of the bacteria organized in a community, as a biofilm. Thus, results obtained in laboratory studies, most likely, may not reflect findings of the same magnitude in the oral cavity.[12]

Results from MIC are difficult to compare with published data since research methodologies are quite different among studies; however, it is possible to verify that the results relate to the information that the MIC of essential oils mouth rinse is between 4 and 32 dilutions of the commercial product.[13]

Haffajee in 2008 studied the MIC of three antiseptics in different bacteria, including *S. mutans*, using a laboratory process that may have changed the alcohol composition of the solution, making it impossible to compare with present results. In addition, Elworthy in 1995 used delmopinol to find the MIC for *Streptococcus* spp. Once again, the methodology was different making it impossible to compare with the present results.

Concerning the inhibition of bacterial growth, the only result with statistical significance in the present study refers to aerobic bacteria. A similar finding was presented by Balbuena, with a significant reduction of anaerobic and aerobic bacteria, for a period of 4 h after the use of an antiseptic mouth rinse;[14] however, in our case, the delmopinol mouth rinse presented a significantly lower inhibitory capacity than the essential oils and chlorhexidine, with in turn had no difference between them. Essential oils and chlorhexidine similarity against oral biofilms are also described in the literature.[15]

Table 2: Mean values, in mm, of the bacteria growth inhibition halos and *P* values

	Essential oils	Delmopinol	Chlorhexidine (0.2%)	*P*
Streptococcus mutans	14.5	15.0	14.8	0.953
Lactobacillus	21.1	21.0	22.9	0.559
Aerobic bacteria	14.0	10.0	12.8	0.028*
Anaerobic bacteria	14.5	11.2	13.8	0.053

*Statistically significant

Table 3: *P* values for the differences between treatments

Comparison between mouth rinses (aerobic bacteria)	mm	*P*
Chlorhexidine versus essential oils	12.8 versus 14.0	0.114
Chlorhexidine versus delmopinol	12.8 versus 10.0	0.044*
Essential oils versus delmopinol	14.0 versus 10.0	0.039*

*Statistically significant

The similarity in results, for inhibition, between the essential oils mouth rinse and chlorhexidine does not match the literature, where an essential oil mouth rinse presents a lower efficacy against dental plaque when compared with chlorhexidine.[16-20]

CONCLUSIONS

From this *in vitro* study, it is possible to conclude that:

1. The MIC of an essential oil and a delmopinol mouth rinse, determined by a disk-diffusion test, of colonies of *S. mutans*, *Lactobacillus*, and aerobic and anaerobic bacteria, are lower than the commercially available concentration of the mouth rinses
2. The ability to inhibit growth of *S. mutans*, *Lactobacillus,* and anaerobic bacteria is similar among essential oils, delmopinol, and 0.2% chlorhexidine. Statistical significant differences are only found for aerobic bacteria, for which delmopinol mouth rinse was significantly less able to reduce bacteria growth than essential oils and chlorhexidine mouth rinses which, in turn, were similar.

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Conflicts of interest
There are no conflicts of interest.

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